

To eat or not to eat—relationship of lichen herbivory by snails with secondary compounds and field frequency of lichens

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Abstract

Aims

The biochemical defense of lichens against herbivores and its relationship to lichen frequency are poorly understood. Therefore, we tested whether chemical compounds in lichens act as feeding defense or rather as stimulus for snail herbivory among lichens and whether experimental feeding by snails is related to lichen frequency in the field.

Methods

In a no-choice feeding experiment, we fed 24 lichen species to snails of two taxa from the Clausilidae and Enidae families and compared untreated lichens and lichens with compounds removed by acetone rinsing. Then, we related experimental lichen consumption with the frequency of lichen species among 158 forest plots in the field (Schwäbische Alb, Germany), where we had also sampled snail and lichen species.

Important findings

In five lichen species, snails preferred treated samples over untreated controls, indicating chemical feeding defense, and *vice versa* in two

species, indicating chemical feeding stimulus. Interestingly, compared with less frequent lichen species, snails consumed more of untreated and less of treated samples of more frequent lichen species. Removing one outlier species resulted in the loss of a significant positive relationship when untreated samples were analyzed separately. However, the interaction between treatment and lichen frequency remained significant when excluding single species or including snail genus instead of taxa, indicating that our results were robust and that lumping the species to two taxa was justified. Our results imply lichen-feeding snails to prefer frequent lichens and avoid less frequent ones because of secondary compound recognition. This supports the idea that consumers adapt to the most abundant food source.

Keywords: acetone rinsing, biodiversity exploratories, defense strategies, gastropoda, herbivore resistance, lichenivory, mollusk grazing, plant–animal interaction

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INTRODUCTION

Biochemical plant defense against natural enemies, especially herbivores, is among the most prominent examples of evolutionary adaptation. Plants evolved many defense strategies against herbivores, including chemical defense by secondary compounds, expressed either constitutively or after induction by herbivore attack (Karban and Baldwin 1997; Kempel *et al.* 2013; Walling 2000). Herbivores, in turn, may adapt to such chemical defenses (Futuyma 2009). Thus, plant–herbivore systems represent some of the best examples of coevolution

(Ehrlich and Raven 1964; Garrido *et al.* 2012; Parachnowitsch and Lajeunesse 2012; Thompson 2009). Interestingly, despite the large interest in plant defenses, their relation to plant biogeography is poorly understood. A plausible assumption is that better defended plants occur more widely. On the other hand, more common plant species may also be more affected by enemies because commonness may facilitate the adaptation of herbivores (Speiser 2001; apparency hypothesis: Feeny 1976; but see Endara and Coley 2011). However, while some studies assessed the links between abundance and defense in single plant species (e.g. Rand 2002), testing this relationships

across a larger set of species has hardly ever been done, and this is especially true for nonvascular plants.

Lichens represent one of the most successful forms of symbiosis occurring in essentially all terrestrial and some aquatic habitats (Lutzoni and Miadlikowska 2009). They share many attributes with plants, including sessile life-form, richness of secondary compounds and being consumed by 'herbivores'. As lichens are consumed by various animals, including gastropods, they represent an important food resource, embedded in complex food webs (Gerson and Seaward 1977; Richardson and Young 1977; Seaward 2008). Gastropods are expected to feed selectively and feeding rates differ strongly among lichen species (Gauslaa 2005; Lawrey 1983; Rundel 1978). Selective feeding of gastropods may well be related to differences in the nutrient content, presence of toxic compounds and also availability of the food resources (Lawrey 1983; Speiser 2001). In fact, lichens are known to produce a wide range of secondary compounds (Huneck and Yoshimura 1996; Rambold *et al.* 2014) deposited as crystals in the cortex or on the outer surface of hyphae in the medulla. These compounds can play different ecological roles, including allelopathic inhibition of competitors, UV protection and defense against pathogens and lichen-feeding animals (Lawrey 1995; Rundel 1978). Furthermore, they can affect lichen palatability and decomposition (Asplund and Wardle 2013). With acetone, carbon-based compounds can be almost completely extracted from desiccated lichen thalli without harming symbionts (Reutimann and Scheidegger 1987; Solhaug and Gauslaa 2001). Thus, lichens are perfect study organisms to manipulate antiherbivore effects of secondary compounds in feeding experiments.

For some of these secondary lichen compounds, the constitutive defensive nature against gastropods has already been documented (Asplund *et al.* 2009, 2010b; Gauslaa 2005; Lawrey 1983). In contrast, some snails are feeding selectively on lichens containing deterrent secondary compounds, suggesting adaptation of some snails to these compounds (Hesbacher *et al.* 1995). Furthermore, some secondary lichen compounds were found to attract and even stimulate lichen-feeding oribatid mites (Reutimann and Scheidegger 1987). So far such an effect has not been shown for any other group of lichen feeders, including the ecologically very important gastropods.

A further limitation of the few previous experiments in which untreated lichens and lichens with experimentally removed secondary compounds had been fed to herbivores is their focus on foliose or fruticose lichens, i.e. those forming leaf-like or shrub-like growth forms. In contrast, crustose lichen species, despite their high species diversity and importance as early colonizers, have never been examined in feeding experiments using the acetone-rinsing technique. Thus, it remains open whether lichen growth form, in addition to secondary lichen compounds, has an impact on feeding preferences.

The distribution, abundance and diversity of lichens depend on various factors including their substrate and habitat specificity, tolerance to land use and environmental pollution (Boch *et al.* 2013c; Purvis *et al.* 2010) and on their colonization, dispersal and establishment ability (Shriver *et al.* 2012; Werth *et al.* 2006). Lichens may reproduce by vegetative propagules, thallus fragments or fungal spores. In addition to wind and water dispersal and exozoochory (Seaward 2008), our recent study showed that all these structures also can be dispersed by snail endozoochory (Boch *et al.* 2011). Endozoochorous dispersal by gastropods is also known from bryophytes, ferns and seed plants (Boch *et al.* 2013a; Türke *et al.* 2012), which might play an important role for their diversity and abundance (Boch *et al.* 2015; Türke *et al.* 2012). However, whether lichen–gastropod interactions including endozoochorous lichen dispersal have an impact on lichen frequency and composition in natural habitats is still unclear.

We present a no-choice experiment feeding thalli of 24 lichen species of different growth form (crustose/noncrustose) to individuals of two common snail taxa and compared feeding rates of untreated lichens with lichens whose secondary compounds had been removed by acetone rinsing. We tested whether (i) chemical compounds in the 24 lichen species acted as feeding defense or rather as feeding stimulus for the two snail taxa, (ii) lichen growth form has an impact on feeding preferences, (iii) effects are consistent across the two snail taxa and (iv) the field frequency of lichen species in the region, where we had sampled experimental snails and lichens, is related to the feeding behavior of the two snail taxa.

MATERIALS AND METHODS

Study system

This study forms part of the Biodiversity-Exploratories project (Fischer *et al.* 2010), with field parts in the biosphere area Schwäbische Alb (southwest Germany; for details, see Boch *et al.* 2013d) and laboratory experiments in Bern (Switzerland; 46°57'12"N, 7°26'42"E).

Vegetation data

During 2007 and 2008, we recorded the presence of lichen species for each type of substrate (bark, dead wood, soil and rocks) occurring up to a height of 2 m in 158 forest plots of 20 m × 20 m. We assessed the frequency of each lichen species as the total number of plots where the particular lichen species occurred. The 158 plots are distributed over an area of ~422 km². Together, the plots comprised different management types and habitat characteristics relevant for lichens and snails: the seven unmanaged forest plots harbored mature, deciduous forests dominated mainly by European beech (*Fagus sylvatica* L.). The 131 age-class forest plots were dominated by European beech (94 plots) and Norway spruce (*Picea abies* (L.) H. Karst.; 37 plots) and had different developmental stages of even-aged structure due to

harvests at 80- to 120-year intervals. The 20 selection forest plots harbored uneven-aged deciduous stands dominated by European beech, in which single or small groups of trees were harvested selectively. Based on a large forest inventory (Boch *et al.* 2013c), the number of our sampled plots is proportional to the frequency of these forest types in this region, thus our sampling resulted in unbiased estimates of regional lichen abundance.

Lichen species

We collected thalli of 24 lichen species from bark of recently felled European beech, large-leaved lime (*Tilia platyphyllos* Scop.) and living blackthorn (*Prunus spinosa* L.) in the Schwäbische Alb near Münsingen (48°23'N, 9°27'E; Table 1). All 24 lichen species are

common across large parts of Central Europe (Smith *et al.* 2009) but their abundances vary strongly among regions and habitats (Boch *et al.* 2013b; Stofer 2006) because of their different ecological requirements (Smith *et al.* 2009). Lichen nomenclature follows Smith *et al.* (2009). We obtained the number and identity of secondary compounds of lichen species from Tanahashi *et al.* (1997, 2003) and Smith *et al.* (2009). Prior to the start of the experiment, lichen material was air dried and then stored at −18°C. We removed foliose and fruticose species from bark and cut out crustose species, only leaving a small piece of bark underneath the lichen shape. Half of the lichen material of each species was leached four times in acetone (100%) for 20 min in each run to extract carbon-based compounds (Solhaug and Gauslaa 2001). This method does not affect the palatability of

Table 1: lichen species with functional categories and habitat description

Lichen species	Growth form	Frequency among 158 plots	Secondary compounds	Collected from
<i>Buellia griseovirens</i>	Crustose	53	Atranorin, norstictic acid and other substances of the stictic acid complex ^a	<i>Fagus sylvatica</i>
<i>Dimerella pineti</i>	Crustose	98	No substances detected by TLC ^a	<i>F. sylvatica</i>
<i>Evernia prunastri</i>	Fruticose	22	Atranorin, evernic acid and usnic acid ^a	<i>F. sylvatica</i>
<i>Graphis scripta</i>	Crustose	93	No substances detected by TLC ^a , graphislacones and 6H-dibenzo[b,d]pyran-6-one ^b	<i>F. sylvatica</i>
<i>Hypocenomyce scalaris</i>	Crustose	6	Lecanoric acid and unidentified substances ^a	<i>Tilia platyphyllos</i>
<i>Hypogymnia physodes</i>	Foliose	70	Atranorin, chloratranorin, physodic acid, physodalic acid, 3-hydroxyphysodic acid and protocetraric acid ^a	<i>F. sylvatica</i>
<i>Lecanora chlarotera</i>	Crustose	129	Atranorin, californin, gangaleoidin and roccellic acid ^a	<i>F. sylvatica</i>
<i>Lecanora expallens</i>	Crustose	46	Thiophanic acid, usnic acid, arthothelin and unidentified substances ^a	<i>F. sylvatica</i>
<i>Lecidella elaeochroma</i>	Crustose	73	Arthothelin, granulysin and 4,5-dichlorolichexanthone ^a	<i>F. sylvatica</i>
<i>Lepraria incana</i>	Crustose	116	Divaricatic acid, nordivaricatic acid and zeorin ^a	<i>F. sylvatica</i>
<i>Melanohalea exasperatula</i>	Foliose	13	No substances detected by TLC ^a	<i>F. sylvatica</i>
<i>Opegrapha rufescens</i>	Crustose	16	No substances detected by TLC ^a	<i>F. sylvatica</i>
<i>Parmelia sulcata</i>	Foliose	89	Atranorin and salazinic acid ^a	<i>F. sylvatica</i>
<i>Pertusaria amara</i>	Crustose	25	Picrolichenin acid and protocetraric acid ^a	<i>F. sylvatica</i>
<i>Pertusaria leioplaca</i>	Crustose	81	Coronatol, constictic acid, norstictic acid and stictic acid ^a	<i>F. sylvatica</i>
<i>Phlyctis argena</i>	Crustose	120	Norstictic acid ^a	<i>F. sylvatica</i>
<i>Physcia adscendens</i>	Foliose	65	Atranorin ^a	<i>F. sylvatica</i>
<i>Pleurosticta acetabulum</i>	Foliose	0	Norstictic acid and related compounds ^a	<i>T. platyphyllos</i>
<i>Porina aenea</i>	Crustose	101	No substances detected by TLC ^a	<i>F. sylvatica</i>
<i>Pseudevernia furfuracea</i>	Fruticose	47	Atranorin and physodic acid ^a	<i>F. sylvatica</i>
<i>Pyrenula nitida</i>	Crustose	34	Unidentified anthroquinones ^a	<i>F. sylvatica</i>
<i>Ramalina farinacea</i>	Fruticose	22	Usnic acid, norstictic acid and salazinic acid or protocetraric acid or hypoprotocetraric acid ^a	<i>F. sylvatica</i>
<i>Ropalospora viridis</i>	Crustose	36	Perlatolic acid, hyperlatic, isohyperlatic and superlatic acids ^a	<i>F. sylvatica</i>
<i>Xanthoria parietina</i>	Foliose	50	Physcion (=parietin) ^a	<i>Prunus spinosa</i>

^aSecondary compounds identified by TLC according to Smith *et al.* (2009).

^bSecondary compounds identified by TLC according to Tanahashi *et al.* (1997, 2003).

lichens (Černajová and Svoboda 2014). As we collected all crustose species from *F. sylvatica* (except *Hypocenomyce scalaris* that was collected from *T. platyphyllos* and showed a positive effect of acetone rinsing on the consumed lichen biomass; Table 1 and Fig. 1), we can exclude that compounds accidentally extracted from the tree bark can explain differences in consumed lichen biomass. Before and after feeding, we weighed lichen thalli after storing them for 2 days in a drying oven at 35°C and another day in silica gel. Thus, we measured the total lichen mass consumed but did not further distinguish whether snails preferred or avoided particular parts of the lichen thallus (e.g. vegetative vs. reproductive parts).

Snail taxa

We collected adult individuals of *Ena montana* (Enidae; $N = 20$) and species from the Clausilidae family (*Balea biplicata*, $N = 8$; *Bulgarica cana*, $N = 1$; *Clausilia dubia*, $N = 1$; *Cochlodina laminata*, $N = 13$; *Cochlodina orthostoma*, $N = 2$; *Macrogastra attenuata*, $N = 1$; *Macrogastra plicatula*, $N = 14$; *Macrogastra ventricosa*, $N = 1$), which, due to their similarity and their close relatedness in terms of habitat type, were considered as representatives of one single taxon for analysis. All snail species are common in temperate Europe, where they occur in the litter layer and on the trunk mainly of deciduous trees in humid forest sites and mossy and rocky limestone habitats (Boschi 2011; Turner et al. 1998). They are known to supplement their diet with fungi and lichens or even to feed exclusively

on lichens and algae (Boschi 2011; Speiser 2001). We sampled snails from trunks of living European beech trees in forests in the vicinity of our plots near Münsingen (48°23'N, 9°27'E) at the same sites where we collected lichens and released them at the sampling site after the experiment. Snail nomenclature follows Turner et al. (1998).

Feeding experiment

We conducted a no-choice experiment, offering always one of four replicates per treatment (untreated vs. acetone rinsed) of moistened thalli of each lichen species (circa 0.2 g dry mass) randomly assigned to one individual of each snail taxon for 48 h (22°C; 14/10 h light/dark cycle; 60% relative air humidity). We assured that each lichen species was offered only once to the same snail individual to avoid food conditioning (as reported by Speiser 2001) or starvation of individuals that were exposed to toxic or deterrent lichen samples. Before the first run, and between runs, snails were individually kept in Petri dishes for 48 h and fed with tissue paper. Sixty-one snail individuals were used in a total number of 384 runs (24 lichen species \times 2 treatments \times 2 snail taxa \times 4 replicate individuals).

Statistical analysis

We examined differences in consumed lichen mass between snail taxa (*E. montana* vs. Clausilidae species), among lichen species and the effect of treatment (presence/absence of secondary compounds) and their interactions with analysis of variance. A mixed model was fitted using individual snails as a

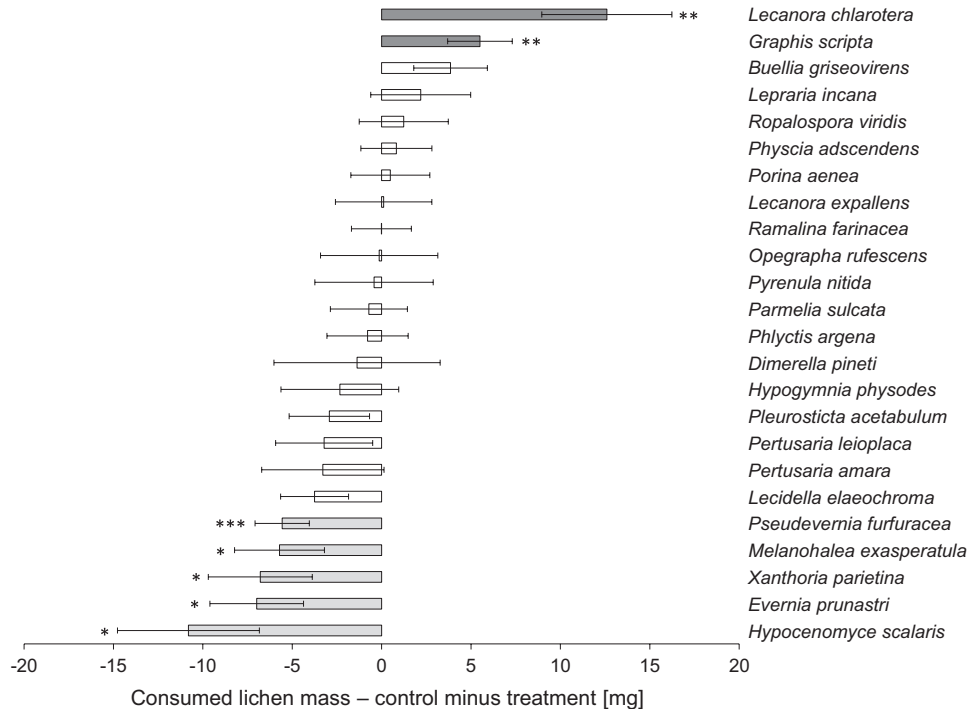


Figure 1: consumed lichen mass of untreated minus acetone-treated replicates (mg) of the 24 lichen species (\pm pooled SD) fed to two snail taxa (*Ena montana* and Clausilidae species) in a no-choice experiment. Lichen species with chemical snail defense are highlighted by light gray and those with chemical snail stimulus by dark gray color. Significant deviation from zero derived by *t*-tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

random effect for significance testing because individual snails were fed in several feeding runs. In addition, to explain variation among lichen species, we included the field frequency of each lichen species and its interaction with the treatment as a contrast indicated in italics to test whether feeding patterns differed between frequent and less frequent lichens. Alternatively, we also tested potential effects of the growth form of lichens and of the number of secondary compounds per lichen species but excluded them as they did not explain variation in consumed lichen biomass (Table 2). Model assumptions were met and therefore no transformation was needed. We also fitted the model by including only well replicated species (*B. biplicata*, *C. laminata*, *M. plicatula* and *E. montana*) and by including genus instead of taxon. This analysis gave qualitatively similar results, indicating that grouping the species of the Clausilidae to one taxon was justified in our case. To test the model's robustness and to assess whether individual lichen species disproportionately affected the results, we refitted the model by omitting a single lichen species at the time. Finally, we calculated the effect size for acetone rinsing for each lichen species as the difference between the means of consumed biomass for acetone-rinsed experimental and control group divided by the pooled standard deviation S :

$$S = \sqrt{\frac{(N^E - 1)(S^E)^2 + (N^C - 1)(S^C)^2}{N^E + N^C - 2}},$$

where S^C and S^E represent the standard deviation and N^C and N^E the sample sizes for control and treatment group, respectively. We then tested for significant deviations from zero

with t -tests. All analyses were done with R, Version 2.8.0 (R Development Core Team 2011).

RESULTS

Consumed lichen mass

The two snails differed slightly in the amount of consumed lichen mass (Table 2), with Clausilidae consuming 23% less than *E. montana*. Acetone-rinsed lichens were consumed slightly, but significantly, more (+15.5%) than untreated lichens were, and this effect was consistent across snail taxa (nonsignificant snail taxon \times treatment interaction; Table 2). There was large variation in the amount consumed among lichen species, which further depended on the snail taxon, as indicated by the significant snail taxon \times lichen species interaction.

Importantly, the consumed lichen mass varied considerably across lichen species, also depending on whether they were acetone-rinsed or not. Single-species analyses showed significant acetone-rinsing effects on consumed biomass for 7 of the 24 species, which is five times more frequent than expected by chance. Snails preferred acetone-rinsed samples of *Evernia prunastri*, *Hypocenomyce scalaris*, *Melanohalea exasperatula*, *Pseudevernia furfuracea* and *Xanthoria parietina* indicating that their secondary compounds acted as chemical defense against snails. Consumed lichen mass did not differ significantly between treatments in *Buellia griseovirens*, *Dimerella pineti*, *Hypogymnia physodes*, *Lecanora expallens*, *Lecidella elaeochroma*, *Lepraria incana*, *Opegrapha rufescens*, *Parmelia sulcata*, *Pertusaria amara*, *P. leioplaca*, *Phlyctis argena*, *Physcia adscendens*, *Pleurosticta acetabulum*, *Porina aenea*, *Pyrenula nitida*, *Ramalina*

Table 2: analysis of variance results for differences in consumed lichen mass between the two snail taxa (*Ena montana* vs. Clausilidae species) and among the 24 lichen species considering their frequency in forests of the Schwäbische Alb region and the treatment effect (presence/absence of secondary compounds)

Source of variation	Consumed lichen mass					
	df1	df2	Sum of squares	Mean squares	F	P
Snail taxa	1	57	510.4	510.3	16.44	<0.0001
Lichen species	23	57	3121.4	135.7	4.36	<0.0001
<i>Frequency</i>	1	22	4.0	4.0	0.03	0.8641
<i>Lichen species</i>	22	57	3117.4	141.7	4.56	<0.0001
Treatment	1	57	124.9	124.9	4.02	0.0496
Interactions						
Snail taxa \times lichen species	23	57	1241.4	54.0	1.74	0.0469
Snail taxa \times treatment	1	57	30.3	30.3	0.98	0.3272
Lichen species \times treatment	23	57	2016.4	87.8	2.82	0.0008
<i>Frequency \times treatment</i>	1	22	667.3	667.3	10.85	0.0033
<i>Lichen species \times treatment</i>	22	57	1352.1	61.5	1.98	0.0204
Lichen species \times snail taxa \times treatment	23	57	932.9	40.6	1.31	0.2053
Individual snail	57	231	1770.0	31.1	1.16	0.2291
Residuals	231		6207.9	26.9		

Indented lines in italics indicate linear contrast to explain variation among lichen species.

farinacea and *Ropalospora viridis*. In contrast, snails preferred untreated samples of *Graphis scripta* and *Lecanora chlarotera* to the acetone-rinsed samples indicating feeding stimulants for snails (Fig. 1).

Relationship with the frequency of lichen species in the field

Overall, we found that the amount consumed in treated and untreated lichen interacted with their frequency in the field, as indicated by a significant lichen frequency \times treatment interaction, whereas in untreated lichens that still contained their secondary compounds, snails consumed more on lichen species that were more frequent in the field. In contrast, in treated lichens from which secondary compounds had been removed, snails consumed more on lichen species that were less frequent in the field (Table 2 and Fig. 2). The exclusion of *L. chlarotera* from the analysis resulted in a nonsignificant relationship between consumed lichen mass and frequency in the field among the untreated lichens. However, the interaction between lichen frequency and the acetone-rinsing treatment remained significant when single lichen species were excluded (all P values < 0.0275), indicating that this result was robust and not driven by an exceptional lichen species. Thus, our results show that the frequency of lichens in the field explained a significant part of the variation in consumed mass and differences in consumed mass among treated and untreated lichens.

DISCUSSION

Snail defense of lichens by secondary compounds

In a group of lichen species, we found no differences in the consumed lichen mass between the control and acetone-rinsing,

i.e. secondary compound removal, treatments. As we did not measure the exact level of reduction of thin layer chromatography (TLC) detectable and nondetectable secondary compounds by acetone rinsing, it may well be that some of these species still contained acetone nondissolvable compounds, which acted as defense against snails. However, we demonstrated that feeding on some lichen species was reduced or even avoided because of their secondary compounds, in accordance with previous studies (e.g. Asplund et al. 2010b; Gauslaa 2005; Lawrey 1983). In contrast to Černajová and Svoboda (2014) who fed individuals of six foliose Parmeliaceae species (untreated lichens vs. lichens whose secondary compounds had been removed) to two gastropod taxa, we found indications for chemical defense in *M. exasperatula*. In addition, we found untreated samples of *G. scripta* to be preferred to acetone-rinsed ones. This is interesting because these two species contain no secondary compounds detectable by TLC (Table 1; but see Tanahashi et al. 1997, 2003 for *G. scripta*, which contradicts general information on secondary compounds of this species, e.g. given by Smith et al. 2009). However, it might be that compounds such as fatty acids (potentially preferred by gastropods, see Lawrey 1983) or pigments (as it was the case for *M. exasperatula*) were removed by acetone rinsing, which acted as defense or feeding stimulus, respectively, for the snails used in our experiment. Lawrey (1984) further mentioned that vegetative parts of the lichen thallus might be less protected against grazing than reproductive structures (e.g. apothecia and perithecia) and that the hymenium (lower part of the apothecium) can contain substances that can prevent grazing. It might thus well be that some lichen species contain acetone dissolvable substances that stimulate snail feeding. In addition, Fröberg et al. (1993) observed on the island of Öland (Sweden) that gastropods consumed more apothecia than perithecia in

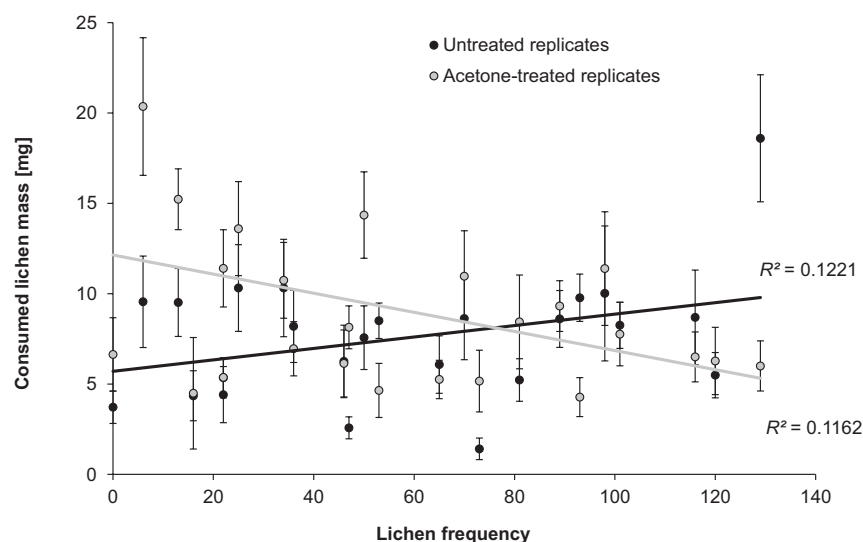


Figure 2: mean consumed lichen mass for untreated and acetone-treated replicates (mg) of the 24 lichen species (\pm SE, $N = 8$) versus the number of plots where these lichen species occurred among 158 forest plots of the Schwäbische Alb region. Trend lines indicate significant relationships with R^2 values weighted by the inverse of the standard error of each observation.

saxicolous lichens. However, as we only measured the total consumed lichen mass, it remains open whether snails preferred or avoided particular parts of the lichen thallus (e.g. vegetative vs. reproductive parts or apothecia vs. perithecia) in our study. This needs further investigation in future studies. Contrary to the findings of Lawrey (1983), the number of secondary compounds in the lichen thallus had no influence on lichen consumption by snails in our experiment.

Differences in lichen herbivory among snail taxa

The differences in lichen herbivory between the two snail taxa of our experiment may well reflect general variation in lichen herbivory among gastropod species, most likely due to adaptation to secondary lichen compounds. This is supported by Gauslaa (2005) who used two snail species of the Helicidae, *Cepaea hortensis* and *Arianta arbustorum*, and Černajová and Svoboda (2014) who used a Clausiliidae snail species, *Cochlodina cerata*, and a Limacidae slug species, *Lehmannia marginata*. In contrast to the snail taxa in our experiment, the Helicidae did not differentiate between acetone-rinsed and control samples of *X. parietina* and preferred acetone-rinsed *H. physodes* and *P. sulcata*, indicating chemical defense in these species (Gauslaa 2005). Moreover, *C. cerata* and *L. marginata* did not differentiate between acetone rinsed and control for *M. exasperatula* and showed ambiguous for *P. sulcata* (Černajová and Svoboda 2014). Combining these results with ours extends our finding that lichen herbivory and adaptation to secondary lichen compounds differs among snail taxa, which is in line with the conclusions of previous studies (Baur *et al.* 1994; Fröberg *et al.* 1993; Hesbacher *et al.* 1995).

Food recognition and secondary compounds

Interestingly, in our study, the snail taxa even preferred untreated samples of two lichen species compared with acetone-rinsed samples. Therefore, at least some secondary compounds in lichens may stimulate snail feeding. This implies food recognition by secondary compounds, as it had been shown for plant-feeding insects (van der Meijden 1996) and for lichen-feeding oribatid mites (Reutimann and Scheidegger 1987). Speiser (2001) mentioned that the slow movement of terrestrial gastropods is costly because of mucus secretion, which may have favored the evolution of a generalized feeding behavior to minimize foraging efforts. The feeding behavior of terrestrial gastropods involves food sampling and learning (e.g. unknown food is first consumed in only small quantities before either rejecting or accepting it), and the final mass consumed depends not only on the nutritional quality such as nutrient content, toxic compounds and palatability but also on the quantity and availability of lichens (Speiser 2001). As a consequence, this may result in completely different diets of the same gastropod species in different habitats and regions. Thus, such a feeding behavior may serve as a plausible mechanism for our finding of an interaction between consumed lichen mass and lichen frequency in the field (Fig. 2). Because the positive relationship between lichen frequency and lichen consumption in

the control treatment occurred only when the most consumed and most frequent lichen (i.e. *L. chlorotera*) was included in the analysis, further studies, e.g. multiple-choice experiments, are needed to test whether lichen-feeding snails generally prefer the common lichen species in different habitats. Nevertheless, we hypothesize that snails might recognize the most frequent lichen species by their secondary compounds, and *vice versa* they avoid feeding less frequent or 'unknown' lichen species. Thus, our data support the idea that abundance of a food source may promote adaptation of the consumer, along the lines of the apparency hypothesis (Feeny 1976).

Mutualistic lichen–snail interactions

In addition to antagonistic herbivorous interactions between gastropods and lichens (e.g. Asplund *et al.* 2010a; Seaward 2008), snails might also interact mutualistically with lichens by promoting fragmentation, proliferation and dispersal of lichen thalli (Boch *et al.* 2011). Therefore, selective grazing by gastropods of frequent lichen species may benefit population growth, whereas less frequent lichen species may contain chemical compounds that reduce snail feeding and, as such, limit opportunities for dispersal. Thus, higher lichen abundance may result from increased herbivory, which may be promoted by improved herbivore adaptation to more frequent lichens, which even could eventually feedback on lichen abundance. Our study calls for field tests of both directions of such mutualistic lichen–snail interactions.

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REFERENCES

- Asplund J, Larsson P, Vatne S, *et al.* (2010a) Gastropod grazing shapes the vertical distribution of epiphytic lichens in forest canopies. *J Ecol* **98**:218–25.
- Asplund J, Solhaug KA, Gauslaa Y (2009) Fungal depsidones an inducible or constitutive defence against herbivores in the lichen *Lobaria pulmonaria*? *Basic Appl Ecol* **10**:273–8.

- Asplund J, Solhaug KA, Gauslaa Y (2010b) Optimal defense: snails avoid reproductive parts of the lichen *Lobaria scrobiculata* due to internal defense allocation. *Ecology* **91**:3100–5.
- Asplund J, Wardle DA (2013) The impact of secondary compounds and functional characteristics on lichen palatability and decomposition. *J Ecol* **101**:689–700.
- Baur A, Baur B, Fröberg L (1994) Herbivory on calcicolous lichens: different food preferences and growth rates in two existing land snails. *Oecologia* **98**:313–9.
- Boch S, Berlinger M, Fischer M, et al. (2013a) Fern and bryophyte endozoochory by slugs. *Oecologia* **172**:817–22.
- Boch S, Fischer M, Knop E, et al. (2015) Endozoochory by slugs can increase bryophyte establishment and species richness. *Oikos*. doi: 10.1111/oik.01536.
- Boch S, Müller J, Prati D, et al. (2013b) Up in the tree—the overlooked richness of bryophytes and lichens in tree crowns. *PLoS One* **8**:e84913.
- Boch S, Prati D, Hessemöller D, et al. (2013c) Richness of lichen species, especially of threatened ones, is promoted by management methods furthering stand continuity. *PLoS One* **8**:e55461.
- Boch S, Prati D, Müller J, et al. (2013d) High plant species richness indicates management-related disturbances, rather than the conservation status of forests. *Basic Appl Ecol* **14**:496–505.
- Boch S, Prati D, Werth S, et al. (2011) Lichen endozoochory by snails. *PLoS One* **6**:e18770.
- Boschi C (2011) *Die Schneckenfauna der Schweiz. Ein umfassendes Bild- und Bestimmungsbuch*. Bern, Switzerland: Haupt Verlag.
- Černajová I, Svoboda D (2014) Lichen compounds of common epiphytic Parmeliaceae species deter gastropods both in laboratory and in Central European temperate forests. *Fungal Ecol* **11**:8–16.
- Ehrlich PR, Raven PH (1964) Butterflies and plants: a study of coevolution. *Evolution* **18**:586–608.
- Endara M-J, Coley PD (2011) The resource availability hypothesis revisited: a meta-analysis. *Funct Ecol* **25**:389–98.
- Feeny P (1976) Plant apparency and chemical defense. In James W, Wallace JM, Mansell RL (eds). *Biochemical Interaction Between Plants and Insects: Proceedings of the Fifteenth Annual Meeting of the Phytochemical Society of North America*, New York, NY: Plenum Press, 1–40.
- Fischer M, Bossdorf O, Gockel S, et al. (2010) Implementing large-scale and long-term functional biodiversity research: the biodiversity exploratories. *Basic Appl Ecol* **11**:473–85.
- Fröberg L, Baur A, Baur B (1993) Differential herbivore damage to calcicolous lichens by snails. *Lichenologist* **25**:83–95.
- Futuyma DJ (2009) *Evolution*, 2nd edn. Sunderland, MA: Sinauer Associates.
- Garrido E, Andraca-Gómez G, Fornoni J (2012) Local adaptation: simultaneously considering herbivores and their host plants. *New Phytol* **193**:445–53.
- Gauslaa Y (2005) Lichen palatability depends on investments in herbivore defence. *Oecologia* **143**:94–105.
- Gerson U, Seaward MRD (1977) Lichen-Invertebrate associations. In Seaward MRD (ed). *Lichen Ecology*. London, UK: Academic Press, 69–119.
- Hesbacher S, Baur B, Baur A, et al. (1995) Sequestration of lichen compounds by three species of terrestrial snails. *J Chem Ecol* **21**:233–46.
- Huneck S, Yoshimura I (1996) *Identification of Lichen Substances*. Berlin, Germany: Springer.
- Karban R, Baldwin IT (1997) *Induced Responses to Herbivory*. Chicago, IL: The University of Chicago Press.
- Kempel A, Nater P, Fischer M, et al. (2013) Plant-microbe-herbivore interactions in invasive and non-invasive alien plant species. *Funct Ecol* **27**:498–508.
- Lawrey JD (1983) Lichen herbivore preference: a test of two hypotheses. *Am J Bot* **70**:1188–94.
- Lawrey JD (1984) *Biology of Lichenized Fungi*. New York, NY: Praeger.
- Lawrey JD (1995) Lichen allelopathy: a review. In Inderjit-Dakshini KM, Einhellig FA (eds). *Allelopathy: Organisms, Processes, and Applications*. Washington DC: ACS Symposium Series, American Chemical Society, 26–38.
- Lutzoni F, Miadlikowska J (2009) Lichens. *Curr Biol* **19**:R502–3.
- Parachnowitsch AL, Lajeunesse MJ (2012) Adapting with the enemy: local adaptation in plant–herbivore interactions. *New Phytol* **193**:294–6.
- Purvis OW, Tittley I, Chimonides PDJ, et al. (2010) Long-term biomonitoring of lichen and bryophyte biodiversity at Burnham Beeches SAC and global environmental change. *Syst Biodivers* **8**:193–208.
- R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rambold G, Elix JA, Heindl-Tenhunen B, et al. (2014) LIAS light – towards the ten thousand species milestone. *MycKeys* **8**:11–6.
- Rand TA (2002) Variation in insect herbivory across a salt marsh tidal gradient influences plant survival and distribution. *Oecologia* **132**:549–58.
- Reutimann P, Scheidegger C (1987) Importance of lichen secondary products in food choice of two oribatid mites (Acari) in an alpine meadow ecosystem. *J Chem Ecol* **13**:363–9.
- Richardson DHS, Young CM (1977) Lichens and vertebrates. In Seaward MRD (ed). *Lichen Ecology*. London, UK: Academic Press, 121–43.
- Rundel PW (1978) The ecological role of secondary substances. *Biochem Syst Ecol* **6**:157–70.
- Seaward MRD (2008) Environmental role of lichens. In Nash TH III (ed). *Lichen Biology*, 2nd edn. Cambridge: Cambridge University Press, 274–98.
- Shriver RK, Cutler K, Doak DF (2012) Comparative demography of an epiphytic lichen: support for general life history patterns and solutions to common problems in demographic parameter estimation. *Oecologia* **170**:137–46.
- Smith CW, Aptroot A, Coppins BJ, et al. (2009) *The Lichens of Great Britain and Ireland*. London, UK: British Lichen Society.
- Solhaug KA, Gauslaa Y (2001) Acetone rinsing—a method for testing ecological and physiological roles of secondary compounds in living lichens. *Symbiosis* **30**:301–15.
- Speiser B (2001) Food and feeding behaviour. In Barker GM (ed). *The Biology of Terrestrial Molluscs*. Wallingford, CT: CABI, 259–88.
- Stofer S (2006) *Working Report ForestBIOTA—Epiphytic Lichen Monitoring*. http://www.forestbiota.org/docs/report_lichens_20060503.pdf (3 December 2014, date last accessed).
- Tanahashi T, Kuroishi M, Kuwahara A, et al. (1997) Four phenolics from the cultured lichen mycobiont of *Graphis scripta* var. *pulverulenta*. *Chem Pharmaceut Bull* **45**:1183–5.

- Tanahashi T, Takenaka Y, Nagakura N, *et al.* (2003) 6H-dibenzo[b,d]pyran-6-one derivatives from the cultured lichen mycobionts of *Graphis* spp. and their biosynthetic origin. *Phytochemistry* **62**:71–5.
- Thompson JN (2009) The co-evolving web of life. *Am Nat* **173**:125–40.
- Türke M, Andreas K, Gossner MM, *et al.* (2012) Are gastropods, rather than ants, important dispersers of seeds of myrmecochorous forest herbs? *Am Nat* **179**:124–31.
- Turner H, Kuiper JGJ, Thew N, *et al.* (1998) *Fauna Helvetica 2: Atlas der Mollusken der Schweiz und Liechtensteins*. Neuchâtel, Switzerland: CSCF.
- van der Meijden E (1996) Plant defence, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. *Entomol Exp Appl* **80**:307–10.
- Walling LL (2000) The myriad plant responses to herbivores. *J Plant Growth Regul* **19**:195–216.
- Werth S, Wagner HH, Gugerli F, *et al.* (2006) Quantifying dispersal and establishment limitation in a population of an epiphytic lichen. *Ecology* **87**:2037–46.